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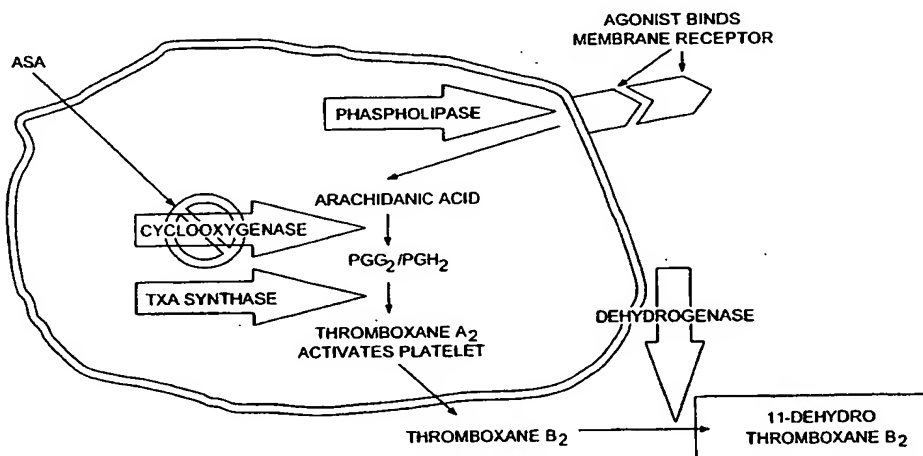
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(54) Title: THROMBOXANE B<sub>2</sub> METABOLITE AND METHODS FOR REGULATING ASPIRIN-RELATED PLATELET ACTION



(57) Abstract: Disclosed are unique methods for identifying the lowest, yet optimal, aspirin doses for patients. These methods are also characterized as having little to no aspirin-related side-effects. These methods may be used pre- as well as post- thrombotic event, and employs a patient's urinary thromboxane B<sub>2</sub> metabolic levels (e.g., 11-dehydrothromboxane B<sub>2</sub>), to identify the patient's platelet activation level. A patient's urinary thromboxane B<sub>2</sub> metabolic level is then used to calculate and appropriate and individualized treatment effective for utilizing platelet activation. Kits for utilizing this technique are also provided. In yet another particular aspect, the invention provides a method for utilizing a random urine sample obtained from a patient to determine whether a patient or particular individual's current dosage of aspirin is providing an adequate and appropriate level of inhibition of platelet activation levels, as compared to inhibition levels observed in individuals not taking aspirin. The figure shows a biochemical pathway in a cell releasing 11-dehydrothromboxane B<sub>2</sub> from thromboxanes B<sub>2</sub>.

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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

1  
**THROMBOXANE B<sub>2</sub> METABOLITE**  
**AND**  
**METHODS FOR REGULATING**  
**ASPIRIN-RELATED PLATELET ACTION**

**RELATED APPLICATION**

The present application is a utility application filing of U.S. Provisional patent application 60/161,462 filed October 25, 1999, to which priority is claimed.

**BACKGROUND OF THE INVENTION**

Aspirin (acetyl salicylic acid) effectively reduces the risk of secondary thrombotic events in individuals who have experienced angina, myocardial infarct, peripheral artery disease, or cerebrovascular ischemia. Aspirin also may reduce the risk of initial thrombotic events in healthy individuals. For this reason, many individuals, through physician prescriptions or self-medication, take aspirin on a regular basis for the primary or secondary prevention of thrombotic disease.

Some important questions remain unanswered. What long-term dosage of aspirin is likely to confer protection from thrombosis while avoiding gastric discomfort or hemorrhagic conditions? What individuals are "resistant" to aspirin, how may aspirin resistance be identified, and what effect does aspirin resistance have on the interpretation of clinical trials?

Several studies suggest that aspirin, at doses between 30 and 325 mg/day, is effective in reducing the incidence of arterial thrombotic events. Physicians customarily prescribe aspirin to prevent myocardial infarction, cerebrovascular thrombotic disease, and vascular death in individuals with stable angina (1), unstable angina (2), myocardial infarction (3), transient cerebral ischemia, peripheral vascular disease, and thrombotic stroke. (4) For patients with prior acute myocardial infarction or stroke, aspirin prevents up to 40 new thrombotic events per thousand treated. (5) Two large randomized trials demonstrate the thrombosis-reducing efficacy of aspirin for patients with acute ischemic stroke, and aspirin is now routinely prescribed in these conditions. (6,7)

Aspirin has been shown to be effective in prevention of heart disease in men with several high risk clinical conditions and both men and women with hypertension. (8,9) The Program on the Surgical Control of the Hyperlipidemias categorized a number of individuals who had a partial ileal bypass surgery as smokers and non-smokers and assigned them to aspirin and non-aspirin arms. Within this population, the overall mortality rate was 45.2% for smokers with no aspirin use and only 10.4% for those who reported even infrequent aspirin use. (10)

#### *Primary Prophylaxis with Aspirin*

Aspirin reduces the incidence of thromboembolic arterial disease in healthy individuals over 50 years of age. (11, 12, 13) In healthy men, aspirin appears to prevent an average of four thrombotic events per thousand subjects treated. In the Physicians' Health Study, healthy men who took 325 mg of aspirin every other day experienced a mean reduction in the incidence of first myocardial infarction of 44.8% compared to those taking placebos. (14) There was a particularly marked reduction of 59.3% for the morning peak of infarction, between 4 and 10 a.m. These findings were not confirmed in the British Doctors' study, however, and additional confirmatory studies are in progress, including the current Women's Health Study. (15)

#### *Aspirin Dosage and Complications*

##### *Hemorrhage and Gastrointestinal Toxicity*

There is a dose-related risk of gastrointestinal bleeding in aspirin therapy, especially in patients who have coagulopathy or who are taking additional anticoagulant therapy such as heparin or warfarin. (16) This is of particular concern in individuals with stomach lesions such as the ulcers associated with *Helicobacter pylori* infection. To avoid hemorrhage, many physicians recommend enteric-coated aspirin, especially if the recommended dosage exceeds 325mg/day. (17) In the British Doctors' Study, where the prescribed randomized dose was 500 mg/day, 20% of aspirin arm participants dropped out due to dyspepsia or constipation, 3.6% experienced bleeding or bruising, and 2.2% had gastrointestinal blood loss. (18)

In the Cardiovascular Health Study, Kronmal, et al found a 1.6x relative risk of ischemic stroke and a 4x relative risk for hemorrhagic stroke for healthy women 65 and older who took aspirin. (19) This study presents self-reported, not randomly assigned, aspirin users and illustrates the hemorrhagic risk of high-dose aspirin in particular disease situations.

For patients who have an elevated risk of thrombosis, the absolute benefit of aspirin prophylaxis clearly outweighs the relatively small risk of bleeding. However, the individuals with no risk factors, aspirin dosages must be carefully monitored to avoid gastric discomfort, gastric hemorrhage, systemic hemorrhage and hemorrhagic stroke.

5 ***Recommendations for Therapeutic Aspirin Usage***

Because of the preponderance of evidence favoring the protective effects of aspirin against arterial thrombosis, millions rely on aspirin prophylaxis daily either by physician's prescription or self-medication. In a review of clinical studies undertaken in the 1980s and 1990s, Hirsh, Dalen, Fuster, et al make the following recommendations: (4)

- 10
- Aspirin is indicated for patients with stable angina, unstable angina, acute myocardial infarction, transient cerebral ischemia, thrombotic stroke, and peripheral arterial disease.
  - A dose of 75 to 100 mg/day should be used chronically for all indications, although an initial dose of 160 to 325 mg should be used in acute settings.
  - 15 • For patients with cerebrovascular disease, a dose of 75 mg/day is effective.
  - Aspirin at 100 mg/day is indicated for patients with prosthetic heart valves who develop systemic embolism while on warfarin.
  - Aspirin is indicated for patients with atrial fibrillation in whom warfarin is contraindicated.

20 There is no recommendation either for or against aspirin usage in normal, healthy adults, and no standards have been established for laboratory monitoring of aspirin's efficacy, a growing concern as clinicians became aware of aspirin resistance and interindividual pharmacokinetic variations.

25 Recent clinical investigations indicate that approximately 10% to 15% of patients on aspirin therapy for prevention of thrombosis have a less than adequate platelet suppression response. (20) Additionally, it is reported that some individuals develop an increasing resistance over time. (21) It is uncertain if this observed resistance is the result of poor absorption, changes in pharmacodynamics, non-compliance, or mechanisms not currently identified. Ridker, et. al. reported that the positive effect of aspirin appears to be altered by

30 underlying inflammation and further suggested that markers of inflammation such as C-reactive protein may delineate those individuals who will respond atypically to aspirin. (22)

**Platelet Activity Studies and Variable Aspirin Response**

Current laboratory measures of platelet activation include the Mielke template bleeding time, aggregometry, lumiaggregometry, the Dade-Behring PFA-100 platelet

function analyzer, and the platelet reactivity test. (23) While the platelet-suppressing property of aspirin clearly affects the results of these procedures in general, individuals' laboratory responses to aspirin therapy are idiosyncratic. Trip et. al. report 46% of patients with positive spontaneous platelet aggregation results suffered a repeat myocardial infarction. (24) This was the first study that paired clinical outcomes with laboratory results. Helgason, et. al. demonstrated that seven of 17 patients with atrial fibrillation achieved only partial inhibition of platelet aggregation when taking 325 mg of enteric coated aspirin per day. Non-compliance had no statistical effect on this study's outcome. Helgason's group further demonstrated that 8.2% of patients with previous ischemic stroke exhibited restoration of platelet aggregation (aspirin resistance) despite escalation of aspirin dosage to, ultimately, 1300 mg/day. (25)

Pappas, et al used a specially designed visual platelet aggregate inspection technique to measure the response to the inhibitory effects of aspirin in 31 healthy young adults. (26) They demonstrated a wide inter-individual variation in response to aspirin that was consistent over 28 days of aspirin ingestion. Mueller, et al performed corrected whole blood aggregometry on patients with intermittent claudication who were taking 100 mg aspirin/day after elective peripheral balloon angioplasty. (27) At any given time, only 40% of males showed complete inhibition of aggregation. Significantly, non-responsive aggregometry results in this study predicted increased risk of reocclusion, leading to the conclusion that aspirin may fail to protect partial-and non-responders from occlusive events.

Grotemeyer, et al, performed whole platelet reactivity tests on 180 internal carotid artery stroke victims given 500 mg of aspirin three times per day. (28) All began with elevated platelet reactivity immediately following stroke. Upon initial aspirin administration, 90% of the subjects demonstrated an immediate suppression of platelet reactivity. However, 60 patients' platelets resumed enhanced platelet reactivity only twelve hours after the initial aspirin dosage. These were termed secondary aspirin non-responders. Over a 24-month period following discharge, 24 (40%) of the secondary aspirin non-responders experienced myocardial infarct, repeat stroke, or vascular death ( $p < 0.0001$ ). Of 114 remaining subjects (six were lost to follow-up), only five (4.4%) suffered these major endpoints. Grotemeyer concluded that early identification of secondary aspirin non-responders is an important step to effective prevention of further thrombotic events in post-stroke patients. In an extensive review of aspirin and platelet laboratory studies, Patrono, et al, commented that 10% to 15% of individuals have a poor initial response or demonstrate progressive resistance to aspirin. No large clinical trials have incorporated laboratory

measures of platelet activation, so the effect of aspirin resistance on clinical outcomes is currently unknown.

Komiya, et al used platelet aggregometry to detect cases of aspirin therapy non-compliance and incorrect dosage. They found that 10% of 159 outpatients' results were outside the diagnostic parameters because of non-compliance and that an additional 2% were confirmed compliant but still had normal aggregometry results. (29) Their study illustrates the necessity for monitoring aspirin therapy in patients who may be suspected of non-compliance.

### **Aspirin Suppresses Platelet Activity**

#### **10 *Agonists Trigger Platelet Activation***

The cyclooxygenase (COX) biochemical activation pathway, as diagrammed in figure 1. This event is essential to normal platelet activation and to the prevention of systemic hemorrhage. (30) COX is also an important activation enzyme in other cells.

Activation begins when a platelet agonists such as ADP, epinephrine, collagen, or thrombin binds to its platelet membrane receptor site. This activates phospholipase A<sub>2</sub>, a membrane-associated enzyme. Phospholipase A<sub>2</sub> frees arachidonic acid, a 20-carbon unsaturated fatty acid, from its supporting membranes phospholipid. Free arachidonic acid is a substrate for the COX pathway. (31)

#### ***The Platelet Cyclooxygenase Pathway***

20 COX, a membrane-associated endoperoxide synthase with two catalytic sites, rapidly modifies the free arachidonic acid in a two-step process. (32) The first catalytic site converts it to the endoperoxide PGG<sub>2</sub>. The Second site, a peroxidase-type site, converts the short-lived PGG<sub>2</sub> to PGH<sub>2</sub>. PGH<sub>2</sub> is then converted by the isomerase action of thromboxane synthase to thromboxane A<sub>2</sub> (TXA<sub>2</sub>), which activates the platelet.

25 TXA<sub>2</sub> is rapidly hydrolyzed to thromboxane B<sub>2</sub> (TXB<sub>2</sub>), a stable plasma product of the COX pathway. TXB<sub>2</sub>, in turn, is converted to a variety of end products, most of which are excreted via the kidney. (33)

#### ***Aspirin Irreversibly Acetylates Cyclooxygenase***

30 Platelets (and other cells) are now known to produce two isoforms of COX-1 and COX-2. (34) COX-1 is a constitutive membrane-bound enzyme that functions in all normal platelets, whereas COX-2 is a cytokine-inducible enzyme that appears in newly produced platelets and in other cells during inflammation.

Aspirin irreversibly acetylates both COX-1 and COX-2 at serine 529, see figure 2. For the COX-1 enzyme, the attached acetyl group sterically hinders arachidonic acid's

access to its reactive site. Acetylation does not appear to hinder the activity of COX-2. The inflammation-induced activity of COX-2 in platelets may account for some cases of aspirin resistance (35), as may pharmacokinetic variations among individuals.

#### *Aspirin Pharmacokinetics*

Aspirin is rapidly absorbed from the stomach and duodenum and is rapidly hydrolyzed to salicylic acid by esterases in the gut, liver, and erythrocytes. Because only aspirin, not salicylic acid, acetylates COX-1 (or COX-2), a significant proportion of acetylation occurs in the presystemic circulation of the gut and liver. (36) Salicylic acid circulates bound to plasma proteins for up to six hours and is cleared by the kidney.

Platelets acetylated during the time of peak aspirin levels lose most of their ability to be activated; as reflected in prolonged bleeding times, reduced aggregometry responses, and diminished TXB<sub>2</sub> production. A single dose of 325 mg aspirin is detectable within minutes using these laboratory assays and the effects remain for six to ten days. Platelets with acetylated COX-1 survive normally and continue to participate in the adhesion reaction. Normal platelet function test results are restored only when a predominant population of new platelets has been released from the bone marrow.

#### *Additional Platelet Activity-Suppressing Substances*

Other non-steroidal anti-inflammatory drugs (NSAIDs) such as dipyridamole, sulphinpyrazone, and ibuprofen act upon COX-1 or other platelet enzymes, but no clinical trials have established antithrombotic properties for these drugs. Ticlopidine, clopidogril, and abiximab exert their antiplatelet activity on membrane receptors. The effects of all these therapeutics may be measured using aggregometry, lumiaggregometry, and plasma, serum, or urine TXB<sub>2</sub> assays.

In addition, many dietary components and supplements have been shown to modify platelet function by unknown mechanisms. These include fish oil (37), vitamin E (38), garlic (39), red wine, and purple grape juice (40). Herbal and dietary supplements may both interfere with or enhance the effect of aspirin on platelets.

Janssen, et al provided healthy volunteers with 3 mg of aspirin per day, a study designed to mimic acetylsalicylic acid levels in certain plants. (41) They showed that serum TXB<sub>2</sub> levels were reduced by 39% compared to placebo.

A number of prospective randomized clinical trials have demonstrated that 50 to 500 milligrams of aspirin per day effectively reduces the risk of primary or secondary arterial thrombotic events in many individuals. Recognition of aspirin's antithrombotic properties



has prompted clinical researchers to focus substantial efforts towards modifying platelet function with not only aspirin but a variety of newly developed platelet-suppressive drugs.

Despite the availability of these new drugs, the use of aspirin for arterial thrombosis prevention continues to increase as public awareness grows. More than 80 billion aspirin tablets are consumed annually in the USA, and more than 37% of the individuals taking aspirin do so to "prevent blood clots". Additionally, individuals who employ alternative medicine practices may consume significant quantities of red wine, purple grape juice, fish oil, vitamin E, garlic, ginkgo biloba, and other substances known to interfere with platelet function.

Numerous reports in the scientific literature detail varied individual responses to aspirin dosages. However, little is reported regarding the potential need to adjust aspirin dosage according to individual biologic response or to a changing response over time. Limited studies correlate the occurrence of thrombotic events with individuals who become "resistant" to aspirin.

Subject	3	8	12	14	17	18	24
% Inhibition (81)	60	78	46	48	70	17	10
% Inhibition (325)	44	40	82	83	46	83	38

Why hasn't aspirin, the drug most widely consumed to modify platelet function, been dosed according to biologic response, in a manner similar to anticoagulants? Because most laboratory tests used to monitor platelet function are time consuming, require expensive equipment (platelet aggregometer), or are traumatic to the patient (bleeding time). In addition, all current assay methods are subject to wide variation in results due to preanalytical and analytical variables, inherent in all *ex vivo* tests that require the patient's platelets.

### SUMMARY OF THE INVENTION

The present invention, in a general and overall sense, provides a method for identifying an optimal minimal aspirin dose for a patient that is specifically tailored to the patient's specific platelet response levels (i.e., to monitor platelet in activation).

The method includes measurement of thromboxane B<sub>2</sub> metabolite levels in the patient to determine the optimum dose for platelet inhibition, with minimum aspirin related side-effects. It is envisioned that the invention, in one aspect, may also be provided in a test kit form. In some embodiments, the kit will be provided together with a solid substrate that is at least partially coated with a material such as an antibody, that is capable of reacting

with a thromboxane B<sub>2</sub> metabolite, such as 11-dehydro TXB<sub>2</sub>. Also included would be a reactor fluid, such as one that would change in color upon exposure to the thromboxane B<sub>2</sub> metabolite.

### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 -- Biochemical pathway in a cell showing release of 11-dehydro thromboxane B<sub>2</sub> from thromboxanes B<sub>2</sub>.

Figure 2 -- Biochemical pathway showing the reaction products, salicylic acid and acetylated cyclooxygenase from cyclooxygenase and acetylsalicylic acid.

Figure 3 -- pg 11 -- dehydro TXB<sub>2</sub>/mg creatinine (x axis) is frequency (y axis)

Figure 4 -- □ = Baseline

■ = Post 81mg

Figure 5 -- □ = Baseline

■ = Post 325mg

Figure 6 -- □ = Baseline

■ = Post 81mg

(Shaded Box) □ = Post 325mg

### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

**EXAMPLE 1****Measuring Platelet Activation*****Platelet aggregometry and in vitro activation***

One may detect and measure *in vitro* platelet activation using aggregometry, which  
5 detects platelet aggregation; lumiaggregometry, which detects both aggregation and platelet-specific secretions; or platelet-activation instrumentation such as the Dade-Behring PFA-100 platelet function analyzer.

***Platelet activation metabolites and in vitro activation***

Plasma, serum, or urine assays of platelet secretions and arachidonic acid pathway  
10 metabolites may be employed in the detection of *in vitro* platelet activation. Assays of plasma or serum TXB<sub>2</sub> are used extensively in platelet function research laboratories where specimen management is carefully controlled, but routine clinical measurement is hampered by the *in vitro* instability of platelets.

***Aspirin dosage and measures of platelet activity***

15 Measurement of aspirin-induced platelet suppression has led to some unexpected findings. Using the arachidonic acid metabolite 12-L-5, 8, 10 – heptadecatrienoic acid (12-HHT) as marker, Beving et. al. measured aspirin's suppression of platelet activity at three dosages, 30, 75, and 150 mg/day. (42) His group demonstrated that, after seven days' treatment, discontinuing the higher dosages triggered a rebound phenomenon. This was  
20 reflected in significantly elevated 12-HHT levels persisting for up to six weeks after stopping aspirin therapy. Further, they demonstrated the rebound to be greater in patients whose baseline levels of 12-HHT were elevated. They concluded that the degree of platelet suppression and ultimate rebound effect could be controlled by determining the pre-aspirin platelet activity via 12-HHT level analysis and by adjusting aspirin dosage accordingly.  
25 The rebound effect did not occur in the 30 mg/day dosage arm.

Buerke et. al. used bleeding times, platelet aggregometry, and serum TXB<sub>2</sub> assays to demonstrate effective aspirin dosages in healthy males. (43) Comparing various dosage combinations, they recommended a loading dose of 300 mg aspirin in combination with 40  
30 mg/day as a maintenance dose to achieve optimum suppression of platelets. Loading dosages of 40 or 100 mg failed to elicit significant changes within two hours of administration.

***The urinary activation marker 11-dehydro thromboxane B<sub>2</sub>***

Whole blood or plasma specimens for platelet metabolite assays require special management because the platelets tend to become activated by changes in temperature.

exposure to non-biological surfaces, and mild mechanical agitation. Aggregometry assays are qualitative and technology-intensive, and bleeding time tests have poor predictive values. Thus, it is necessary to locate a metabolite formed *in vivo* from the products of platelet activation. (45)

5       Hepatocyte 11-hydroxy thromboxane dehydrogenase acts upon plasma TXB<sub>2</sub> to produce 11-dehydro TXB<sub>2</sub>. (46) The plasma half-life of 11-dehydro TXB<sub>2</sub> is 45 minutes, and plasma levels remain in the nanogram range, as it is rapidly cleared by the kidney. (47) The urine concentration of 11-dehydro TXB<sub>2</sub>, however, is plentiful and, as platelets appear to be its only source, proportionally reflects platelet activity within the previous twelve  
10   hours. (46) Urine levels of 11-dehydro TXB<sub>2</sub> are frequently elevated in atherosclerosis, the chronic phase following stroke, transient ischemic attack, intracerebral hemorrhage, and atrial fibrillation. (48) Further, 11-dehydro TXB<sub>2</sub> levels are typically decreased in aspirin therapy, even in cases of atherosclerosis, myocardial infarction, and atrial fibrillation.

      Assays for 11-dehydro TXB<sub>2</sub> have intra- and interassay CVs of  $\leq 10\%$  and do not  
15   cross-react with TXB<sub>2</sub>, 2, 3-dinor TXB<sub>2</sub>, nor other cyclooxygenase metabolites. (49).

### EXAMPLE 2

#### **11-dehydro TXB<sub>2</sub> in Randomly Collected Urine From Aspirin and Non-Aspirin Donors**

Urine 11-dehydro TXB<sub>2</sub> was assayed in random urine specimens to:

- 20
  - Establish a reference range in non-aspirin users.
  - Determine whether aspirin therapy is related to levels below the reference range.
  - Detect aspirin resistance in individuals taking aspirin therapy.

#### **Materials and methods**

##### **25   11-dehydro TXB<sub>2</sub> reference range and results in aspirin treatment**

      Random urine specimens were collected from 65 individuals who had avoided aspirin for at least two weeks and from 45 individuals who were taking 81 or 325 mg/day by prescription. Each specimen was assayed for 11-dehydro-TXB<sub>2</sub>. Specimens were assayed at two dilutions using acetylcholinesterase-linked enzyme immunoassay; the results were  
30   tested for parallelism and averaged. Urine creatinine was assayed using the Jaffe picrate reaction. To normalize for urinary output, results were expressed in pg 11-dehydro TXB<sub>2</sub>/mg creatinine. Results of all populations were compared using the student t-test. Within-day variation is 30% and day to day variation 20%.

***Effect of aspirin on 11-dehydro TXB<sub>2</sub> in healthy non-aspirin users***

In a crossover study, twenty-four healthy individuals who had avoided aspirin for a minimum of ten days collected random baseline urine specimens. They then took one 81 or 325 mg tablet at 8:00 AM and collected a second urine specimen 24 hours after the aspirin was taken. Following a 2-week washout period dosages were reversed and another baseline and 24-hours post-aspirin specimen was collected.

**Results*****Reference range and aspirin values***

Individuals taking 81 to 325 mg/day aspirin exhibit significantly lower levels of 11-dehydro-TXB<sub>2</sub> than those not taking aspirin. See Table 1.

**Table 1**

	<b>Aspirin therapy</b>	<b>Aspirin non-users</b>	<b>p-value</b>
<b>N</b>	45	65	
<b>Mean</b>	523	2080	<0.00001
<b>SD</b>	514	1360	

A decision point of 100 pg 11-dehydro-TXB<sub>2</sub>/mg creatinine yields both false negative and false positive rates of 10%, the best achievable combination, as shown in Table 2. Aspirin effect is ruled in at a decision point of 800 pg/mg or less and ruled out of 1000 pg/mg. Figure 3 is a histogram that illustrates the clear separation between the non-aspirin and aspirin population. Of the aspirin users, six individuals (13%) had results above the decision point and two exceeded the aspirin effect rule-out point of 1000. These six may be aspirin-resistant, and are being observed closely for potential thrombotic events.

**Table 2**

	<b>Decision level in pg/mg</b>	<b>False Positive rate for aspirin effect</b>	<b>True Positive Rate for Aspirin Effect</b>	<b>True Positive Rate for aspirin effect</b>	<b>False Negative rate for aspirin effect</b>
<b>Aspirin rule-in ≤ 800 pg/mg</b>	600	0.0%	75.6%	100.0%	24.4%
	700	0.0%	80.5%	100.0%	19.5%
	800	0.0%	80.5%	100.0%	19.5%
	900	1.8%	85.4%	98.2%	14.6%

<b>Recommended</b>	1000	10.5%	90.2%	89.5%	9.8%
<b>Decision point</b>	1100	17.5%	90.2%	82.5%	9.8%
	1200	24.6%	95.1%	75.4%	4.9%
	1300	29.8%	97.6%	70.2%	4.9%
	1400	33.3%	97.6%	66.7%	2.4%
<b>Aspirin rule-out ≥ 1500 pg/mg</b>	1500	35.1%	100.0%	64.9%	0.0%
	1600	42.1%	100.0%	57.9%	0.0%

### EXAMPLE 3

#### *Effect of aspirin on the 11 dehydro TXB<sub>2</sub> level of healthy non-aspirin users*

Initiation of aspirin therapy causes mean reduction of 68% for 81 mg/day and 76% for 325 mg/day as illustrated in figures 4 and 5. There was no significant difference between the 81 mg/day and 325 mg/day suppression levels.

An immunoassay has been investigated here that measures the effect of aspirin on platelet function. A stable metabolite of the platelet activation process, 11-dehydro-TXB<sub>2</sub>, can be measured in random urine, bypassing the need for *ex vivo* platelet function. Utilizing this assay we have been able to show a significant difference between individuals taking 81 or 325 mg of aspirin and individuals not taking aspirin. Values equal to or less than 800 pg 11-dehydro-TXB<sub>2</sub>/mg creatinine indicates that aspirin usage is sufficiently inhibiting COX-1 activity. Aspirin users with levels over 1000 pg/mg appear to be resistant and not achieving optimal platelet inhibition, as demonstrated in figure 3. Utilizing these decision point criteria, we define 13% of individuals as potential non-responders, coinciding with other published reports.

The present findings demonstrate that the range of non-aspirin results is broad and appears bimodally distributed. The higher peak represents increased platelet activation.

From the crossover study, seven individuals (30%) had a less than 50% response to either 81 mg or 325 mg of aspirin. Of these, three demonstrated less 11-dehydro TXB<sub>2</sub> excretion at 81 mg than at 325 mg. However, one individual (subject 24) did not demonstrate 50% reduction to 81 mg or 325 mg of aspirin suggesting aspirin resistance. The inhibitory contribution of dietary and lifestyle habits should always be taken into consideration when interpreting data and may have contributed to these results. Further, urine metabolite interference, exercise, and hormonal variation may be responsible for a 20-30% within-day and day-to-day variation.

Aspirin antithrombotic therapy is long-term therapy. The urine 11-dehydro TXB<sub>2</sub> essay is a readily available means for evaluating individual response to aspirin. It is useful for detecting aspirin resistance and for prescribing and monitoring alternate therapies. The assay is also useful in monitoring patient compliance and determining the lowest aspirin dose that produces effective COX-1 inhibition while avoiding aspirin's unpleasant, sometimes dangerous side effects.

Using the 11-dehydro TXB<sub>2</sub> assay as the measure of platelet activity, we recommend clinical trials that 1) further compare biologic response to aspirin's affect on clinical outcomes, 2) evaluate whether dosage adjustment according to COX-1 inhibition will reduce thrombotic events while limiting untoward side effects, and 3) determine whether the influence of inflammatory process, reflected by abnormal C-reactive protein levels, are associated with aspirin resistance.

Aspirin has been proven to prevent secondary arterial thrombosis in a variety of conditions, and may be used as defined here to prevent primary heart attacks and strokes to those who are at risk. Proper monitoring of aspirin therapy will lead to more accurate dosing, prevention of side effects, and the management of aspirin resistance.

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the composition, methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents, which are both chemically and physiologically, related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

#### EXAMPLE 4

The concept of utilizing 11-dehydrothromboxane measurements to determine individuals in whom aspirin or other platelet modifying compounds is not having a significant platelet inhibiting effect may have several additional applications.

The antigen antibody assay currently performed by conventional ELISA technique could also be performed using a point of care instrument. The test method would have to be

modified such that the reaction could take place and the end point be determined within the confines of the point of care instrument.

Additionally, it may be possible to adapt the assay for patient self testing utilizing "dip stick" technology similar to that used for glucose.

#### EXAMPLE 5

##### *Urinary Thromboxane B<sub>2</sub> Metabolite Monitors the Anti-platelet Action of Aspirin*

Concern for side effects and laboratory evidence for aspirin "resistance" in up to 20% of individuals make it essential to establish a simple, effective laboratory monitoring system for aspirin's antithrombotic efficacy. The present example demonstrates the utility of the invention for the purpose in human patients. 11-dehydrothromboxane B<sub>2</sub>, (11-de-H-TXB<sub>2</sub>), a stable metabolite of platelet activation was measured in random urine specimens from 65 people who were not taking aspirin and 45 who were taking at least 81 mg/day. The means for non-aspirin users was 2080 pg 11-de-H-TXB<sub>2</sub>/mg creatinine, and for aspirin users was 523, p. <0.0000.1. Receiver-operating characteristic analysis yields an optimum decision point of 1000 pg 11-de-H-TXB<sub>2</sub> in random urine may be used to document aspirin's platelet-suppressive effects and to identify individuals who may not achieve antithrombotic benefits.

#### REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

1. Juul-Møller S., Edvardsson N. Jahnmatz B, et al, Lancet 1992; 340: 1421.
2. The RISC Group, Lancet 1990; 336: 827.
3. ISIS-2 Collaborative Group; ISIS-2, Lancet 1988; 2:349.
4. The Dutch TIA Study Group, N. Engl. J. Med. 1991; 325: 1261.
5. Hirsh J. Dalen J.E., Fuster V., Harker LB, Patrono C., Roth G., Chest 1995; 108 Suppl.: 247S.
6. International Stroke Trial Collaborative Group, Lancet 1997; 349: 1569.
7. CAST (Chinese Acute Stroke Trial) Collaborative Group, Lancet 1997; 349: 1641.
8. The Medical Research Council's General Practice Research Framework, Lancet 1998; 351: 233.
9. Hansson L. Zanchetti A., Carruthers S.C., et al., Am Heart J. 1995; 129:656.
10. Fitch L.L., Buchwald H., Matts J.P., Am Heart J. 1995; 129:656.



11. Antiplatelet Trialists' Collaboration, BMJ 1994; 308: 81.
12. Hennekens CH, Peo R. Hutchison GB, et al., N. Engl. J. Med. 1988; 318: 923.
13. Mason J.E., Stampfer M.J., Colditz G.A., et al., JAMA 1991; 266:521.

**WHAT IS CLAIMED IS:**

1. A method for determining a minimum optimum aspirin dose for inhibiting platelet activation in a patient treatment regimen comprising:

measuring a level of thromboxane B<sub>2</sub> metabolite in a patient test sample to provide a baseline thromboxane B<sub>2</sub> metabolite level;

determining a level of platelet activation in a urine sample from the patient from whom the test sample was obtained to provide a baseline platelet activation level;

comparing the level of thromboxane B<sub>2</sub> metabolite in the test sample and the amount of platelet activation in the urine sample; and

providing a series of aspirin doses to the patient of 80 mg aspirin, a 200 mg aspirin and of 300 mg aspirin and obtaining a test sample and a urine sample after each dosage; and

identifying the aspirin dosage level that results in a thromboxane B<sub>2</sub> metabolite level of less than 800 mg thromboxane B<sub>2</sub> metabolite, wherein the aspirin dosage level that decreases platelet activation 90% to 95% relative to the baseline platelet activation level is the minimum optimum aspirin dose for inhibiting platelet activation.

2. The method of claim 1 wherein the thromboxane B<sub>2</sub> metabolite is 11-dehydrothromboxane B<sub>2</sub>.

3. The method of claim 1 wherein the patient treatment regimen is a treatment regimen for a post-thrombotic event patient.

4. The method of claim 1 wherein the patient treatment regimen is a treatment regimen for a patient diagnosed as having had ischemia, intracerebral hemorrhage, arterial fibrillation, cardiac catheterization, lupus, or coronary angioplasty.

5. The method of claim 1 wherein the test sample is a urine sample.

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6. The method of claim 1 wherein the test sample is a blood sample.

7. The method of claim 1 wherein the test sample is a plasma sample.

8. A kit for monitoring blood platelet activation comprising:

a substrate capable of attaching thereto an antibody capable of binding to a thromboxane B<sub>2</sub> metabolite; and

a color-indicator liquid material capable of reacting with an antibody that binds to a thromboxane B<sub>2</sub> metabolite.

9. The kit of claim 8 further defined as comprising an instruction sheet.

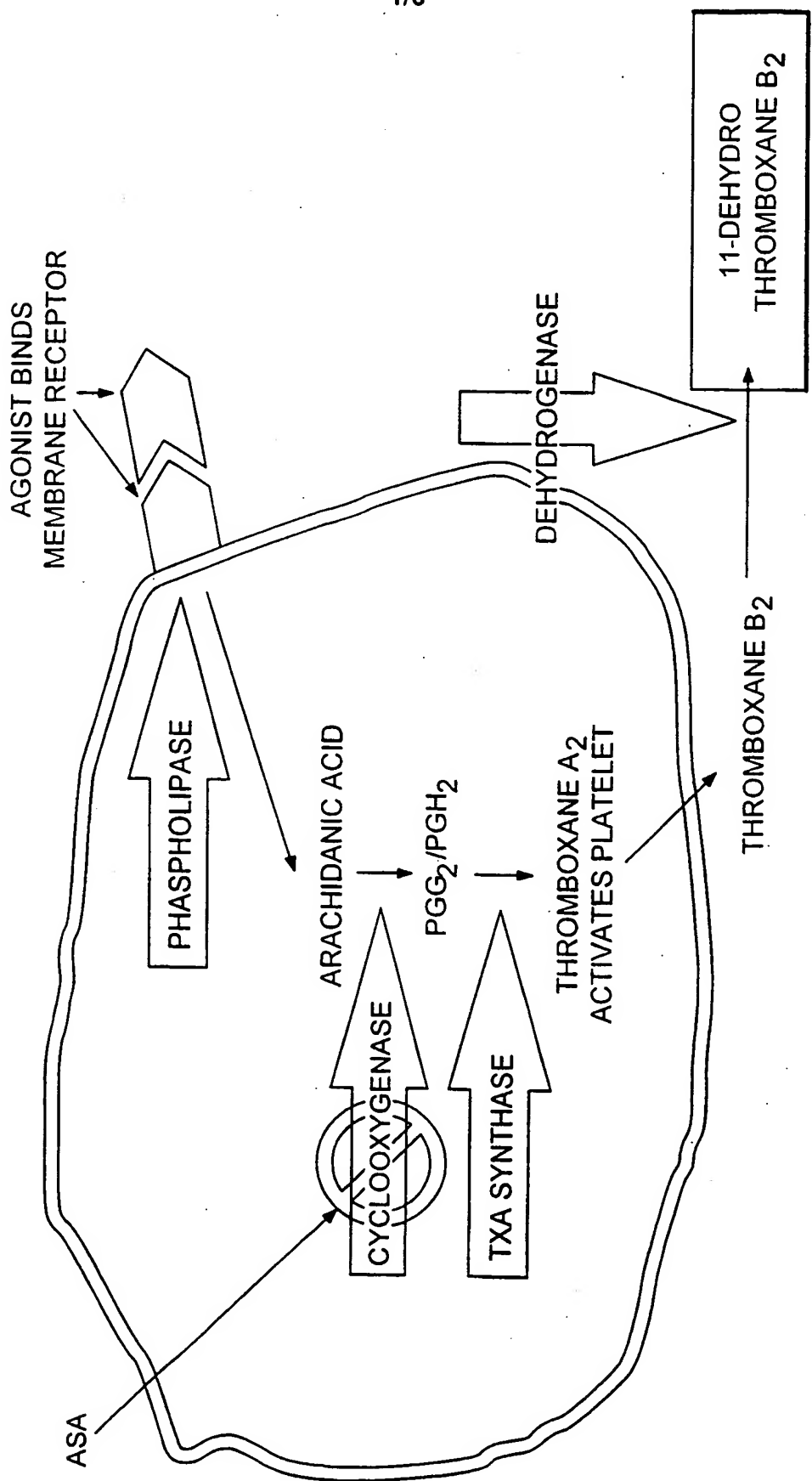


FIG.1

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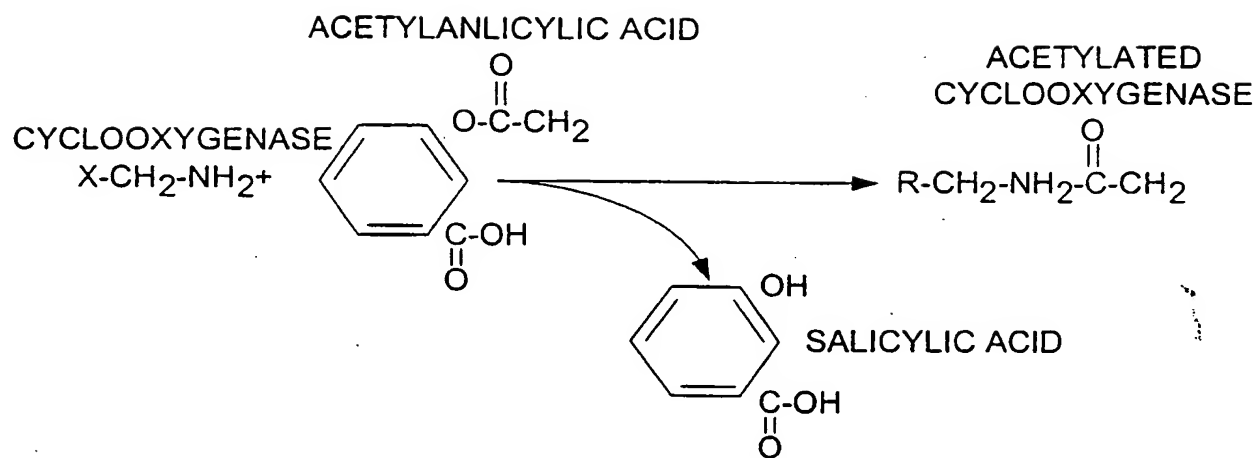


FIG.2

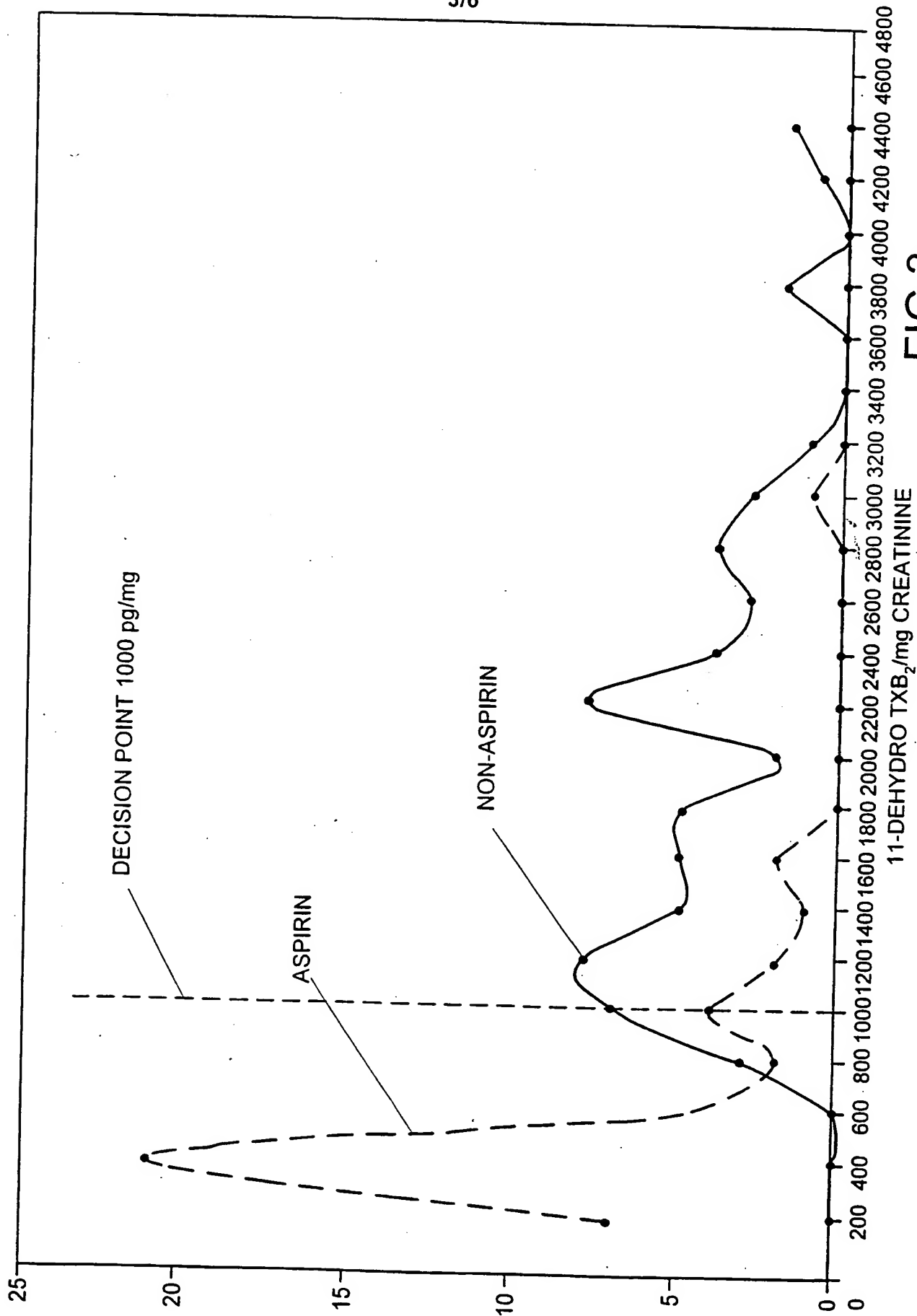


FIG.3

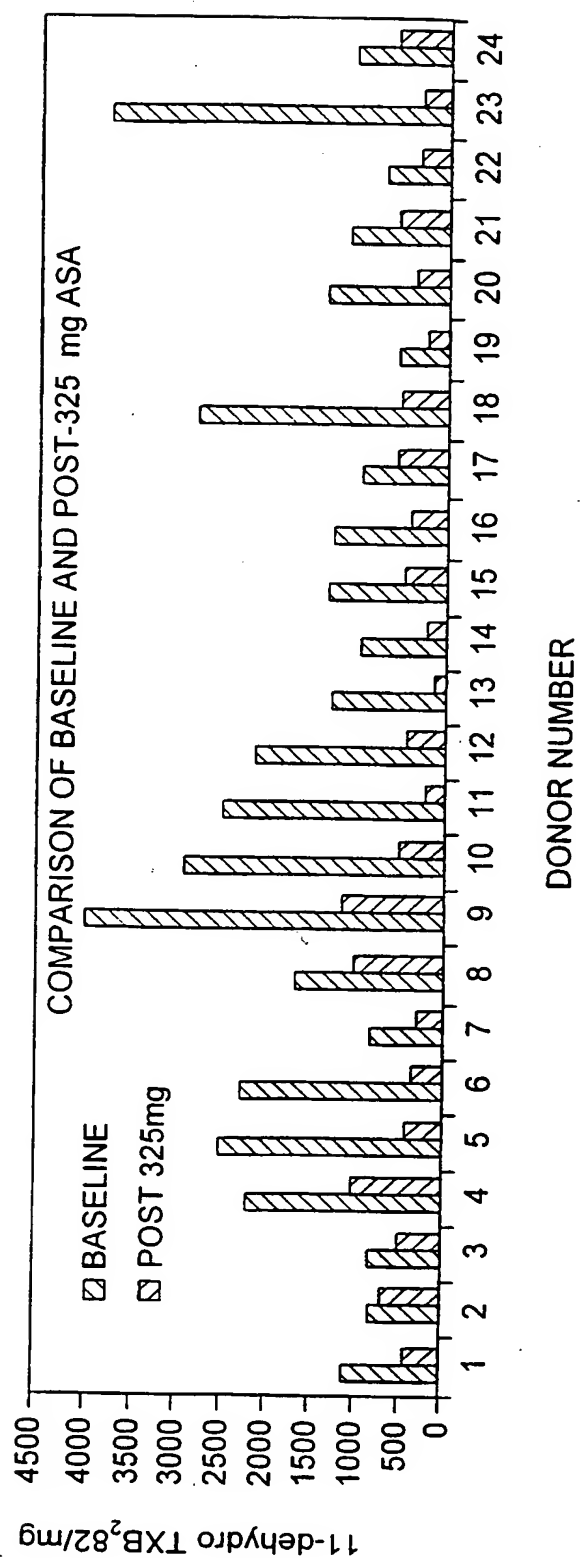


FIG.5

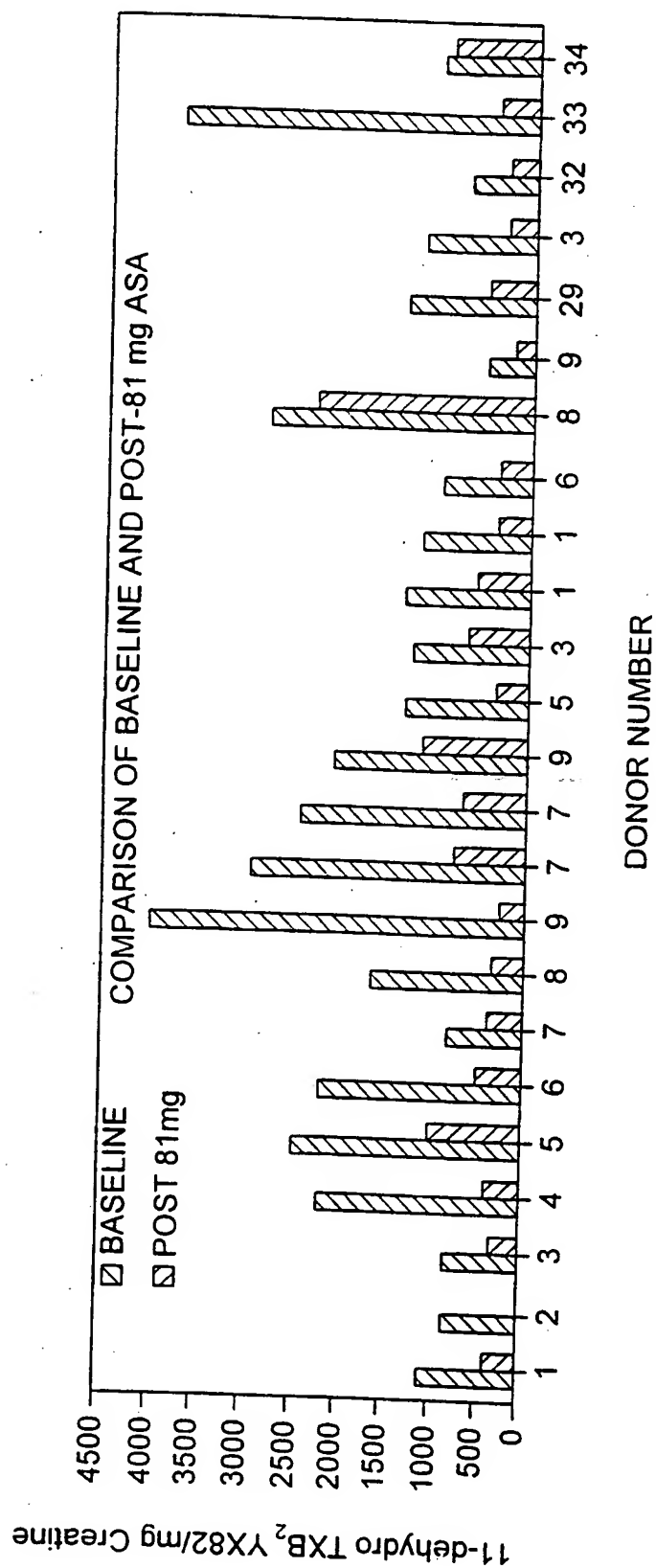


FIG.4



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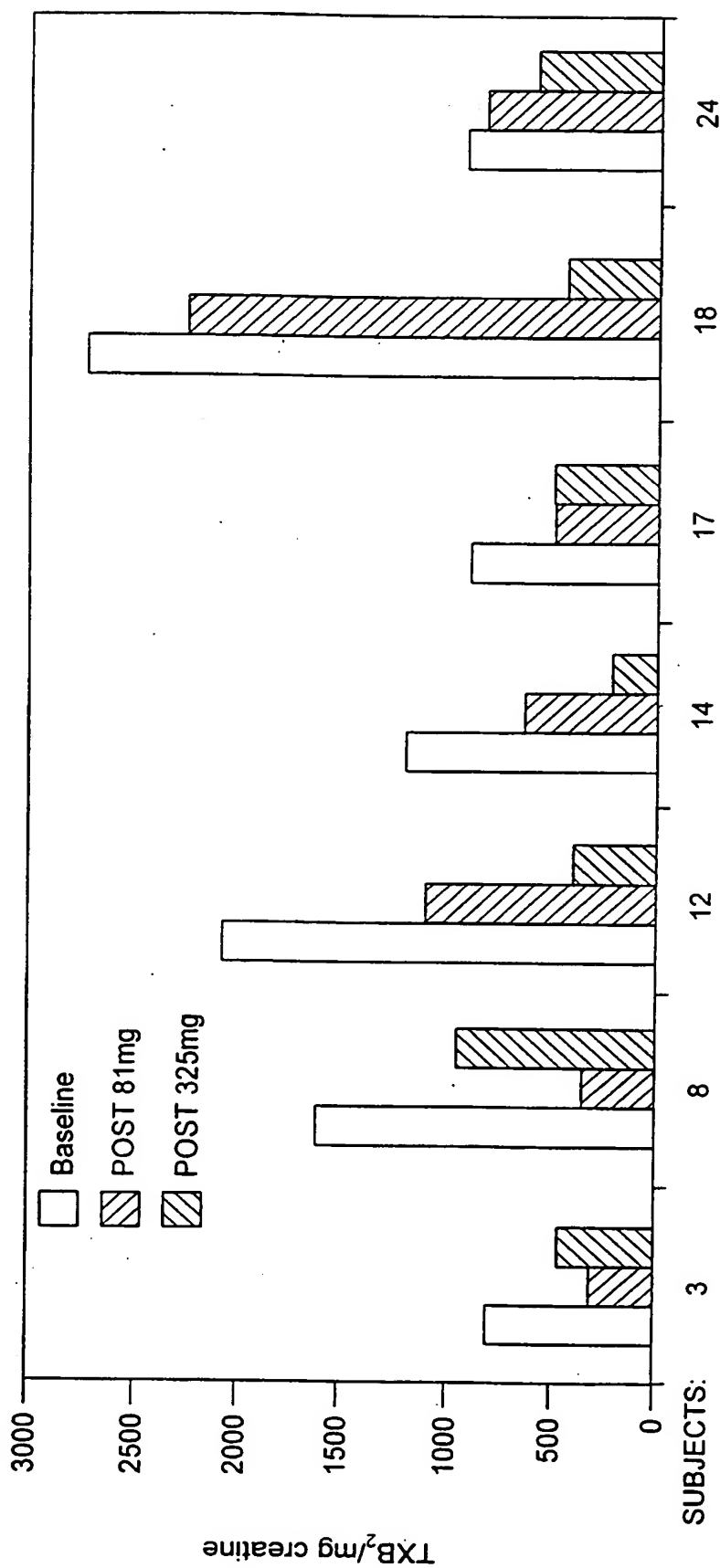


FIG.6

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/19727

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12Q 1/00; G01N 33/53

US CL : 435/4, 975

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/4, 975

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
noneElectronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
WEST: platelet, aspirin, urine, thromboxane

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	US 5,932,226 A (ORDMAN) 03 August 1999, abstract, column 8, lines 18-22 and column 16, lines 20-68.	1-9
A	US 5,427,913 A (SHAW et al) 27 June 1995, column 8, lines 1-68.	1-9

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

## Special categories of cited documents:

- |   |  |
|---|--|
| *A* document defining the general state of the art which is not considered to be of particular relevance  | *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention  |
| *B* earlier document published on or after the international filing date  | *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone   |
| *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| *O* document referring to an oral disclosure, use, exhibition or other means  | *Z* document member of the same patent family  |
| *P* document published prior to the international filing date but later than the priority date claimed  |  |

Date of the actual completion of the international search

24 AUGUST 2000

Date of mailing of the international search report

18 SEP 2000

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